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Surface Topography of Adults and Eggs of *Gnathostoma doloresi* (Nematoda: Spirurida) from Wild Boars (*Sus scrofa leucomystax*)

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ABSTRACT: As few works were found on surface morphology of *Gnathostoma doloresi* Tubangui, 1925, adult specimens and the eggs of this species were examined by scanning electron microscopy. Worms had a subglobular head-bulb with 1 pair of anterior lateral lips. The head-bulb was armed with 7–9 rows of cephalic hooks. Multidigitate cuticular spines were spaced unevenly on transverse cuticular striations on the anterior half of the body. The lengths of the spines were variable with tridentate spines longer than bidentate spines. The posterior half of the body was covered densely with long unidentate spines. Labial papillae and amphids, cervical papillae, caudal papillae, small papillae, and phasmids were also present on the bodies. Eggs recovered from the uteri of female worms were covered with circular pits of comparatively equal size and depth.

KEY WORDS: *Gnathostoma doloresi,* ultrastructure, scanning electron microscopy, adult worms, eggs.

Gnathostoma doloresi Tubangui, 1925, was first reported from a pig in the Philippines. Since that discovery, the endemic area of this parasite has been expanded to include most of southeast Asia and Oceania. Adult worms are normally found in shallow nodules on the stomach wall of pigs and wild boars, the main reservoir hosts for this parasite. Human infections with the advanced third-stage larvae of *G. doloresi* have recently been identified in Japan. This species of gnathostome is currently recognized as an important cause of clinical disease (Ogata et al., 1988; Nawa et al., 1989).

The morphology of adult specimens and larval stages of *G. doloresi* has been described in detail by light microscopy (Tubangui, 1925; Miyazaki, 1950, 1954; Ishii, 1956; Chiu, 1959; Dissamarn et al., 1966; Tada, 1968; Lin and Chen, 1988). More detailed studies of the second-stage, and early and advanced third-stage larvae of *G. doloresi* from cyclops and snakes, respectively, have been done by scanning electron microscopy (SEM) (Koga and Ishii, 1987; Imai et al., 1988; Koga et al., 1989). Observations of adult worms and eggs by SEM have been reported, but the entire outer surface of the worms was not examined (Ishii and Tokunaga, 1970; Sakaguchi et al., 1985; Imai et al., 1989). The present study was designed to examine the surface of adult specimens and eggs of *G. doloresi* in greater detail.

Materials and Methods

Adult specimens of *Gnathostoma doloresi* were collected from the stomachs of naturally infected wild boars (*Sus scrofa leucomystax*) from Kyushu, Japan. Six worms, including males and females, were washed in a glass vial with several changes of tap water and finally rinsed with physiological saline. The worms were fixed with 10% formalin for 7 days, soaked in running tap water overnight to remove the fixative, rinsed with distilled water, and then postfixed in 1% osmium tetroxide for 4 hr. During the postfixation, the specimens were cut transversely into 6 pieces to facilitate observations by SEM. The tissue was then dehydrated with an ethanol series and critical point dried with a Hitachi HCP-2 critical point dryer. After coating with gold in an ion sputter coater (JEOL FC-1100), the specimens were examined with a JEOL JSM-U3 scanning electron microscope operated at 15 kV. All measurements are given in micrometers.

Results

Adult specimens of *Gnathostoma doloresi* are elongate cylindrical nematodes that are covered with evenly spaced rows of transverse striations (Fig. 1). Adult male and female worms examined in this study had a hemispherical head-bulb armed with 7–9 transverse rows of cephalic hooks (Figs. 1, 2). The slender hooks measured about 16 in length and had tapering points (Fig. 3). The mouth was located in the center of the head-bulb and had 1 pair of ellipsoidal lateral lips (pseudolabia). Labial papillae occurred in pairs on each lip and measured about 13 in diameter. Each labial papilla was comprised of fused double papillae (the cephalic and outerlabial papillae). An amphid was present between each pair of papillae (Fig. 4). The body was covered entirely with cuticular spines that originated from the transverse striations. One pair of balloonlike cervical papillae measuring about 20 × 15 in size was located laterally near the 20th striation (Fig. 5). Four pairs of mammiform caudal papillae were located on the ventral surface of the tail of male

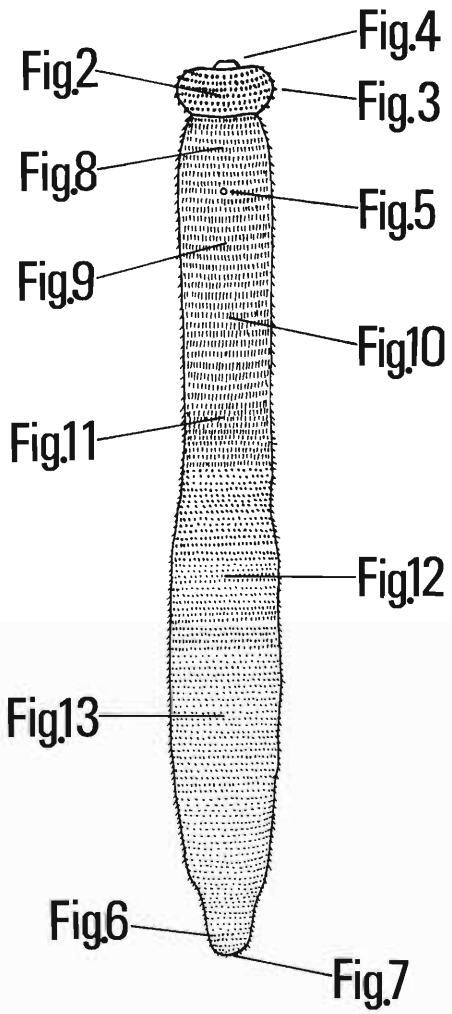


Figure 1. A schematic diagram of an adult *Gnathostoma doloresi*. Lines identify areas of the worm that are illustrated by specific figures.

specimens. An additional 4 pairs of small, dome-shaped papillae, including a pair of cloacal papillae, were located between the larger papillae. The cloacal papillae marked the bilateral edges of the anal opening and were difficult to distinguish at low magnifications (Fig. 6). Slightly elevated bilateral circular phasmids measuring 10 × 12 in size were located at the posterior end of female worms (Fig. 7). These phasmids were not covered with spines.

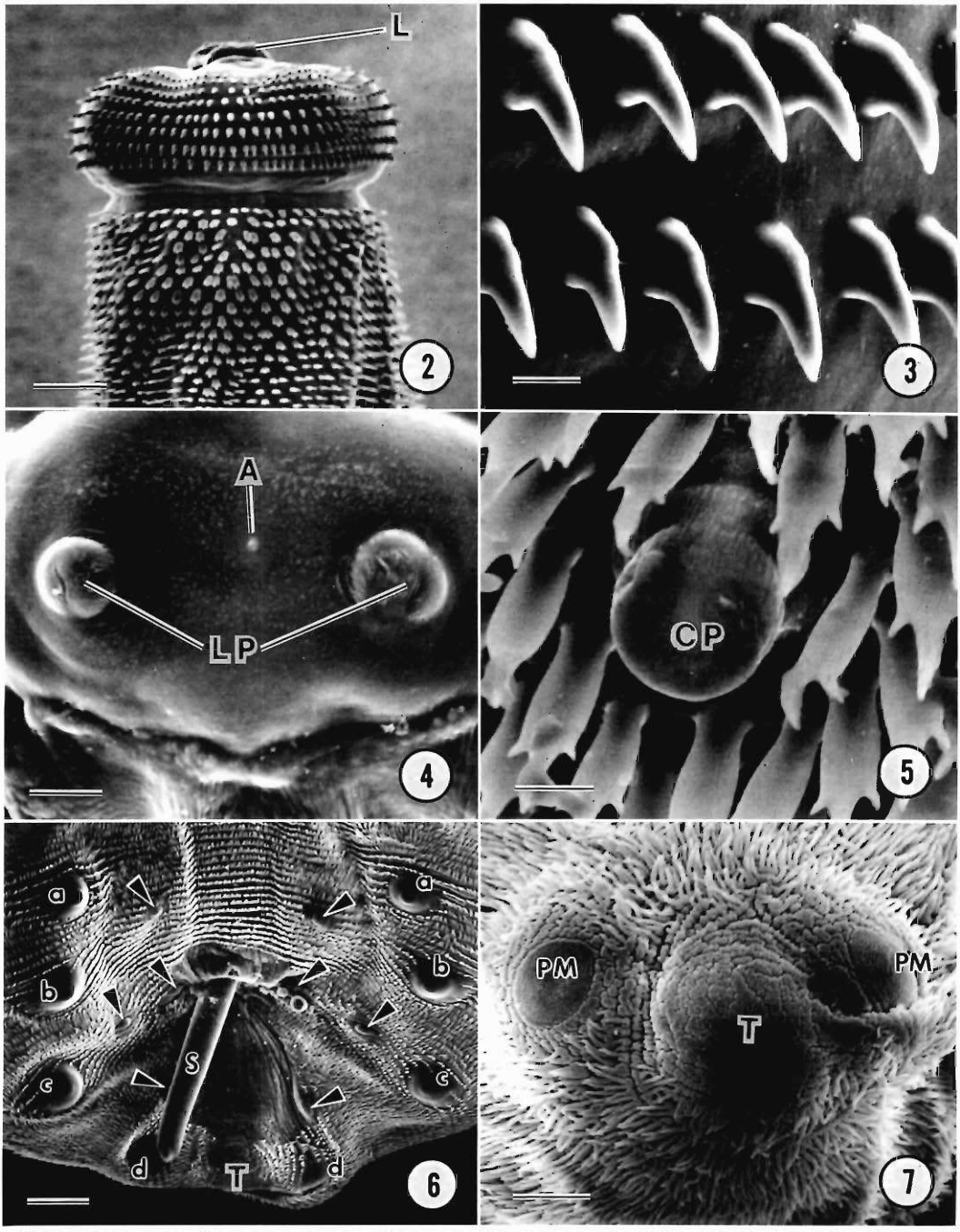
Spines were more variable in shape on the anterior half of worms. Those located immediately behind the head-bulb were broad and stumpy, measured about 17 × 15 in size, and had 5–6 teeth (Fig. 8). Spines became longer and changed in shape as distance from the anterior end of the worm increased, eventually becoming tridentate and reaching sizes of 50 × 6 on the anterior quarter of the body (Fig. 9). These tridentate spines merged gradually into bidentate spines measuring about 40 × 5 in size (Fig. 10) and eventually into unidentate spines (about 40 × 3.5) slightly anterior to the mid-body (Fig. 11). The unidentate spines decreased in length to 15–30 and became more slender and hairlike towards the posterior end of the worms (Figs. 12, 13). The tail of male worms was covered densely with small spines, but the anal opening was surrounded by a spineless area (Fig. 6). Except for the terminal projection and the area of phasmids, the posterior end of female worms was covered densely with hairlike spines (Fig. 7).

Fertilized uterine eggs of *G. doloresi* were ellipsoidal and measured about 40 × 20 in size. The eggs had a pair of opercula and were covered with round pits of uniform depth that were approximately 6 in diameter (Figs. 14, 15).

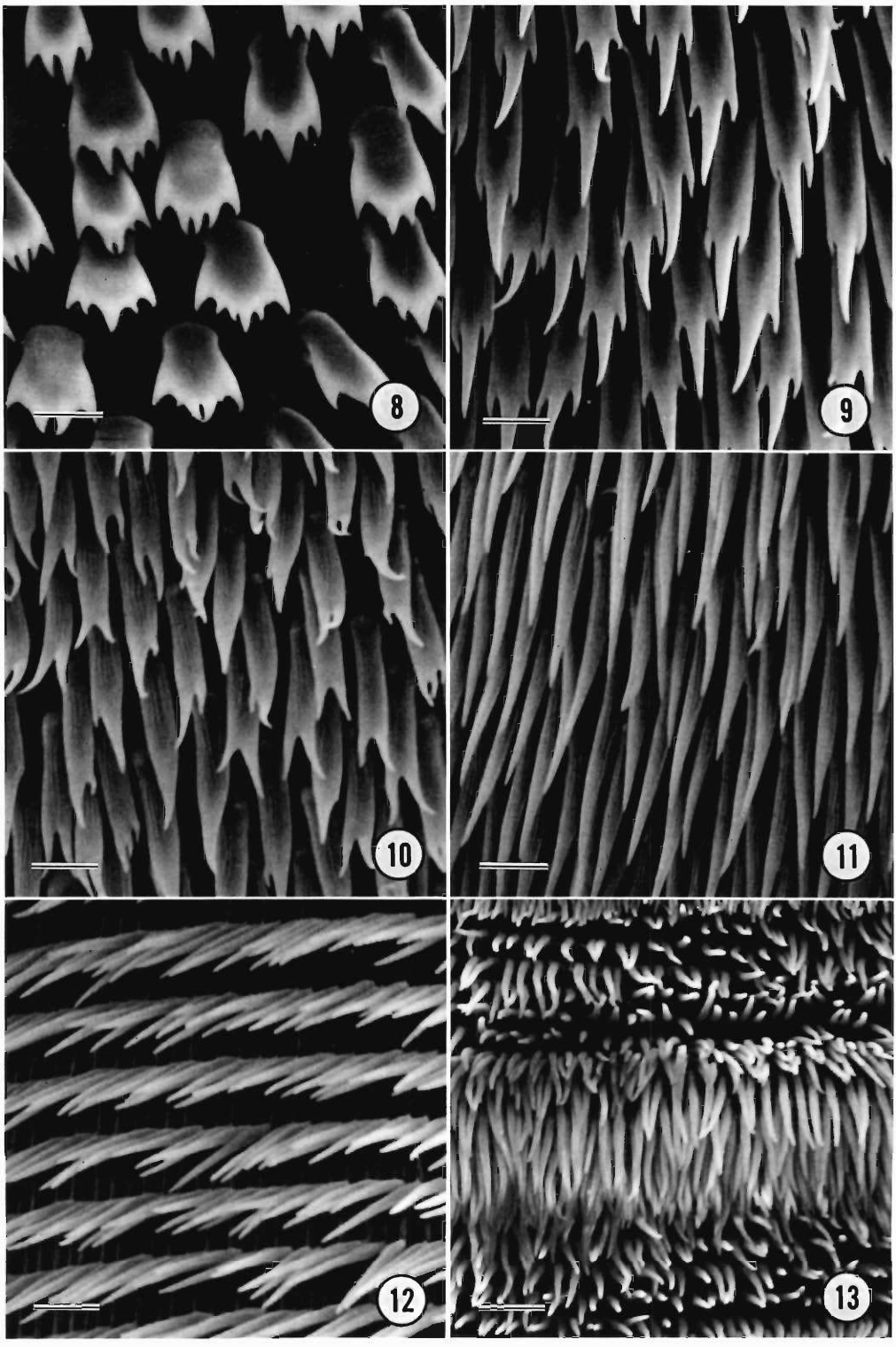
Discussion

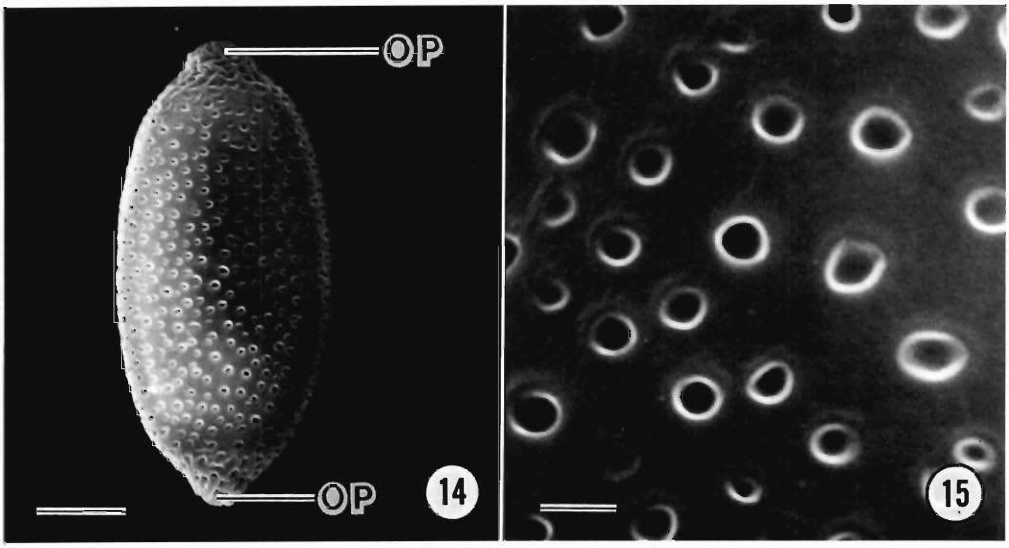
Previous studies of adult specimens of *Gnathostoma doloresi* by SEM provided few details about the location and morphology of papillae. Sakaguchi et al. (1985) did not describe these structures and Imai et al. (1989) only illustrated a pair of cervical papillae. We were able to demonstrate the presence of labial papillae, an amphid, cervical papillae, caudal papillae, small papillae, and phasmids on the adult specimens. The locations of these structures were almost identical to those in other species of gnathostomes (Koga and Ishii, 1981; Koga et al., 1984; Huang et al., 1986; Scholz and Ditrich, 1990). We were unable to demonstrate the excretory pore and posterior body papillae in our specimens of *G. doloresi,* but suspect that they were covered by the abundant, hairlike body spines of this nematode. The mammiform caudal papillae and dome-shaped small papillae on the ventral surface of the tail end of male worms, and the circular, spineless phasmids that occur on the tail ends of female worms, are similar to those that have been described in specimens of *Gnathostoma hispidum* Fedtschenko, 1872 (Koga et al., 1984; Huang et al., 1986).

Miyazaki (1960) conducted a detailed study of the spines of *G. doloresi* by light microscopy and showed that the body is covered entirely by long, slender spines. Sakaguchi et al. (1985) and Imai et al. (1989) demonstrated by SEM that the num-



Figures 2–7. Scanning electron micrographs of adult *Gnathostoma doloresi*. 2. Lateral view of the head-bulb. The bulb has 8 transverse rows of hooks. L, Lip. Scale bar = 80 μm. 3. Hooks located on the head-bulb are unidentate and have sharp tips. Scale bar = 7 μm. 4. Frontal view of the head-bulb. A pair of dome-type labial papillae (LP) and an amphid (A) are visible on 1 of the 2 semicircular pseudolabia. Scale bar = 10 μm. 5. Balloon-shaped cervical papilla (CP) are located on the anterior surface of the body. Scale bar = 7 μm. 6. Four pairs of dome-type caudal papillae (a–d) are present on the ventral side of the male tail. Another 4 pairs of smaller dome-type papillae (arrowheads) are more medially located. S, Spicule; T, Terminal projection. Scale bar = 30 μm. 7. The extreme end of the female tail has 2 bare phasmids (PM) which are elevated slightly above the tegument. The terminal projection (T) has no spines. Scale bar = 10 μm.





Figures 14, 15. Eggs of *Gnathostoma doloresi*. 14. Two opercula (OP) are located at opposite ends of the egg. Scale bar = 7 μm. 15. The surface of the eggshell has many round pits of relatively equal size and depth. Scale bar = 1 μm.

ber of teeth in these spines decreased as distance from the anterior end increased. Structures associated with the tail end of male and female worms were either unrecognizable or not described. Observations from our study on the spacing of spines were most similar to those of Miyazaki (1960), Sakaguchi et al. (1985), and Imai et al. (1989). As seen by SEM, spacing of spines of *G. hispidum* is very similar to that of *G. doloresi*. Spines of *G. hispidum* change size and shape between the anterior quarter and one-third of the body. Around the anterior quarter regions are found the stumpy spines (about 47 × 26 in size) having 5–10 teeth, and they progressively increased in size to about 105 × 12 bearing 3 teeth with the middle 1 markedly elongated at the anterior one-third region. Then the spines abruptly changed shapes to those with 2 denticles. Spines with 1 denticle followed the 2-denticle ones posterior to the anterior one-third body region. These 1-toothed spines entirely covered the rest of the body. Spines at about the mid-body measured 35–65 × 2 and at the posterior extremity about 25–35 × 2 (Koga et al., 1984). Spines of *G. hispidum* seem to be much longer than those of *G. doloresi* in comparable regions.

Early study of the eggs of *G. doloresi* by SEM found ellipsoidal pits in the eggshell (Ishii and Tokunaga, 1970). These structures were more rounded in our specimens, although some pits were oval in shape. The shape of these pits appears to be characteristic for the eggs of *G. doloresi*. The pits resemble those that are found in eggs of *G. hispidum*, but are different from those of *Gnathostoma spinigerum* Owen, 1836, and *Gnathostoma nipponicum* Yamaguti, 1941 (Zaman, 1987; Koga and Ishii, 1981) whose pits have irregular shapes and depths.

To obtain clear, crisp, and concise observations of the location and morphology of spines, papillae, amphids, and phasmids on male and female specimens of *G. doloresi* is very difficult using only light microscopy. There are no descriptions of the cervical papillae and eggshell pits by light microscopy. Our present results may aid in the identification of the adults and eggs of this species of nematode.

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Figures 8–13. Scanning electron micrographs of cuticular spines of *Gnathostoma doloresi*. Depending on location, spines on the surface of adult worms differ in size and shape. Scale bars = 10 μm. 8. Spines immediately behind the head-bulb have 5 or 6 teeth and are broad and stumpy. 9. Slender tridentate spines reach the greatest lengths. 10. Bidentate spines located on the anterior one-third of the body. 11. Unidentate spines are located posterior to bidentate spines and extend to the posterior extremity. 12, 13. Fine, eyelashlike spines cover the posterior half of the body.

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